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The Role of Rubella-Immunoblot and Rubella-Peptide-EIA for the Diagnosis of the Congenital Rubella Syndrome During the Prenatal and Newborn Periods

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Rubella infection during the first trimester of pregnancy can cause the congenital rubella syndrome (CRS). Patients with CRS were shown to have a decreased humoral and cellular immunity. It is not known whether asymptomatic newborns who had experienced intrauterine infection with rubella virus (RV) differ in their antibody response from newborns with CRS. In this study we compared both groups for a difference which might be a useful diagnostic criterion for CRS during the prenatal and newborn periods. We used the nonreducing Rubella-Immunoblot and the Rubella-IgG-Peptide-Enzyme Immunoassay (EIA) to determine the antibodies directed to rubella proteins E1, E2 and C. The results showed that only newborns with CRS who had experienced RV infection during the first 12 weeks of gestation showed significantly reduced levels of antibodies directed to both the linear RV E1 epitope (SP 15) and the topographic RV E2 epitope. Asymptomatic newborns infected mostly later than week 10 of gestation showed normal levels of antibodies. These data suggest that the lack of antibody response in CRS is linked to the immaturity of the fetal immune system during the first trimester of gestation. Rubella-IgG-Peptide-EIA and Rubella-Immunoblot should be used additionally for CRS diagnosis in the prenatal/newborn periods. These results may have an impact on the early treatment of late-onset symptoms of CRS patients. ***J. Med. Virol. 51:280–283, 1997.*** © 1997 Wiley-Liss, Inc.

KEY WORDS: rubella; congenital rubella syndrome; rubella diagnostic methods; prenatal and newborn diagnostic methods; immunoblot; synthetic peptide enzyme immunoassay

INTRODUCTION

Rubella virus (RV) infection during the first trimester of pregnancy can cause the congenital rubella syndrome (CRS). CRS symptoms may include damage to the embryogenic heart, eye and ear, and may also result in late-onset symptoms such as diabetes mellitus.

Since the introduction of rubella vaccination programs, the number of CRS cases has declined remarkably [Centers for Disease Control, 1991]. However, RV infections during pregnancy still occur. Therefore it is necessary to improve further the diagnosis of CRS during the prenatal as well as newborn periods. Improved diagnosis could support better decisions about the pregnancy and about the early treatment of CRS symptoms of newborns. For this reason we evaluated the Rubella-IgG-Peptide-EIA and the nonreducing Rubella-Immunoblot for investigations of prenatal and newborn sera. Both tests are based on the detection of antibodies in patient sera directed to the structural proteins E1, E2 (envelope proteins) and C (capsid protein) of RV. We tested the sera of newborns (CRS and asymptomatic patients) after intrauterine RV infection looking for a possible serological difference between the two groups that might be helpful in the diagnosis of CRS.

PATIENTS AND METHODS

We examined the sera of 18 intrauterine infected newborns, 10 of whom had CRS and eight of whom were born asymptomatic. CRS patients had been exposed to RV during 1–12 weeks of gestation. Asymptomatic patients had been RV exposed mostly after

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TABLE I. Rubella-IgG-Peptide-EIA Results for 10 CRS Newborns, 8 Asymptomatic Newborns, and 15 Postnatally Infected Patients

CRS newborns		Asymptomatic newborns		Postnatally infected newborns	
Patient no.	SP 15 $E1_{208–239}$	Patient no.	SP 15 $E1_{208–239}$	Patient no.	SP 15 $E1_{208–239}$
23	**0.355**	33	0.110	108	**0.796**
24	**0.726**	34	**0.467**	109	**0.998**
25	0.145	35	**0.474**	110	0.033
26	0.001	36	**0.784**	111	0.204
27	0.057	37	**0.593**	112	–0.122
28	**0.380**	38	**0.446**	113	0.119
29	**0.332**	39	**2.116**	114	**0.947**
30	0.016	40	0.156	115	0.111
31	0.101			116	0.156
32	0.120			117	0.216
				118	0.213
				119	0.208
				120	–0.038
				121	**1.699**
				122	**0.369**

The sera were examined for antibody response using the RV synthetic peptide (SP) number 15 as antigen. CRS patients had been exposed to RV during 1–12 weeks of gestation. Asymptomatic patients had been RV exposed mostly after week 12 of gestation (except one case each during weeks 9, 10, and 12). Results are given as extinction values, from which a blank value was subtracted. Bold face indicates numbers which are above the cut-off level for the SP. Except for patient 24 extinction values for SP 15 are less frequent and weaker in CRS newborn patients than in asymptomatic newborns. The asymptomatic newborn patients did not differ significantly from the postnatally RV infected patient group.

week 12 of gestation (except one case each during weeks 9, 10, and 12). All were examined around the same time after the exposure to RV (20 to 61 weeks, in one case 15 months). For comparison we also examined sera of postnatally infected patients (n = 31), including the mothers of the newborns. Before testing with Rubella-Immunoblot and Rubella-IgG-Peptide-EIA, all patient sera were analysed using standard rubella diagnostic methods to confirm the clinical diagnosis.

The Rubella-IgG-Peptide-EIA is based on the determination of antibodies in serum directed to synthetic peptides (SP) which correspond to important linear epitopes of the structural proteins of the RV antigen. Results are given as an extinction value. The test was carried out as described [Wolinsky et al., 1991]. In addition, we determined cut-off levels for the extinction of the SP by testing 29 sera which had <4 IU/ml rubella IgG antibodies (Behring test kit) and no rubella IgM antibodies (Sorin test kit).

The nonreducing Rubella-Immunoblot (as described by Zhang et al., 1992) measures IgG antibodies in serum directed to topographic epitopes of E1, E2 and C of the RV. The resulting bands (E1-, E2-, C-band) were analysed subsequently by densitometry for quantitative comparisons using the software program WinCam 2.1 (Cybertech).

Finally the results of the Rubella-Immunoblot and the Rubella-IgG-Peptide-EIA were analysed for statistical significance by using the Mann-Whitney-U-test (alpha 0.5%, 2-tailed P).

RESULTS

Rubella-IgG-Peptide-EIA

The results of the Rubella-IgG-Peptide-EIA showed a difference between SP 15 antibody levels of the CRS newborns and the asymptomatic newborns. The CRS patients as a group had significantly less frequent and lower SP 15 extinction values than the asymptomatic newborns patient, 24 being the exception. The Mann-Whitney-U-test showed $P = 0.047$. Results are shown in Table I. The extinction values are a measure of the amount of antibodies in serum. Thus, most CRS newborns had fewer antibodies directed to linear epitopes of RV E1 protein ($E1_{208–239}$) than the asymptomatic patients.

We examined 22 mothers of CRS and asymptomatic newborns for their SP extinction values. No significant difference was found. Results are shown in Table II.

Nonreducing Rubella-Immunoblot

In the majority of CRS newborns the E2-bands were found less frequently and had weaker densitometric values than in asymptomatic newborns. The difference between both groups was significant ($P = 0.02$). Their E1- and C-bands did not differ significantly ($P = 0.81$; $P = 0.45$). Hence, in the sera of CRS newborns mostly there were fewer antibodies directed to E2 protein than in the sera of asymptomatic newborns. The levels of antibodies directed to E1 and C proteins did not differ. For results see Table III.

DISCUSSION

It was shown that CRS patients have a reduced level of antibodies directed to the linear RV E1 protein [Mitchell et al., 1992; Mauracher et al., 1993]. In those studies patients with postnatally acquired RV infection served as the control group. We were interested in using intrauterine RV infected asymptomatic newborns as a control group. We compared their antibody responses with those of CRS newborns. The results using

TABLE II. Rubella-IgG-Peptide-EIA Results for Mothers of CRS Newborns (n = 9) and Asymptomatic Newborns (n = 13)

Patient no.	SP 2 $C_{14–19}$	SP 8 $C_{9–29}$	SP 10 $C_{64–97}$	SP 14 $C_{9–25}$	SP 15 $E1_{208–239}$	SP 21 $E2_{31–55}$	SP 22 $E2_{51–75}$	SP 23 $E2_{81–105}$	CP1 $C_{11–29}$ $E1_{221–239}$
RV infected mothers of asymptomatic newborns									
1	−0.031	−0.146	−0.084	−0.064	0.312	NT	−0.056	−0.047	−0.004
2	0.297	**0.240**	0.220	0.038	**0.747**	NT	0.140	0.095	**0.410**
3	−0.132	−0.122	−0.082	−0.116	0.126	NT	−0.078	−0.140	−0.026
4	0.025	−0.051	−0.079	−0.027	−0.030	−0.069	−0.051	−0.060	0.227
5	−0.188	0.067	−0.145	−0.171	−0.160	0.077	0.013	0.328	0.027
6	0.069	0.049	0.100	0.040	0.140	0.003	0.145	0.044	0.303
7	0.637	−0.166	−0.030	−0.152	−0.137	**0.702**	−0.059	−0.147	−0.144
8	−0.008	0.069	0.056	0.053	0.286	−0.116	0.114	0.061	0.042
9	−0.192	−0.366	0.012	0.065	−0.187	−0.212	−0.179	−0.165	0.150
10	0.156	0.113	**0.589**	0.079	**0.544**	0.077	0.114	0.074	**0.912**
11	−0.164	**−0.211**	−0.062	−0.299	0.181	−0.202	−0.217	**0.856**	**0.913**
12	0.549	0.093	0.152	−0.004	**0.338**	**0.801**	−0.001	0.110	**0.807**
13	**2.086**	**0.297**	0.217	0.118	0.164	**2.247**	0.067	0.119	0.308
RV infected mothers of CRS newborns									
14	0.014	−0.184	−0.099	−0.052	**0.799**	−0.234	0.040	0.079	0.056
15	**0.923**	−0.008	0.131	−0.018	0.090	**0.867**	−0.046	−0.088	0.133
16	0.091	−0.029	0.020	−0.007	**0.379**	−0.074	0.015	0.019	0.038
17	−0.315	−0.382	−0.313	−0.367	0.058	−0.382	−0.301	−0.382	−0.233
18	−0.064	−0.329	−0.350	−0.096	**0.336**	−0.502	−0.055	−0.266	−0.266
19	0.508	−0.010	0.364	0.001	0.076	**0.811**	0.019	0.027	0.275
20	0.075	0.050	−0.003	0.023	**0.454**	−0.106	0.109	0.034	0.114
21	−0.470	−0.579	−0.572	−0.398	0.095	−0.579	−0.328	−0.084	−0.302
22	0.098	**0.318**	0.033	−0.094	0.051	0.003	0.003	0.010	−0.012

The mothers' sera were examined for antibody response using eight different RV synthetic peptides (SP) and one RV chimeric peptide as antigen. Results are given as extinction values, from which a blank value was subtracted. Boldface indicates numbers which are above the cut-off level for that SP. Extinction values are similar in both groups of mothers for all SP, even SP 15. NT, not tested.

TABLE III. Nonreducing Rubella-IgG-Immunoblot Results for Intrauterine Rv Infected CRS Newborns (n = 10) and Asymptomatic Newborns (n = 8)

	CRS newborns				Asymptomatic newborns		
Patient no.	E2-band	E1-band	C-band	Patient no.	E2-band	E1-band	C-band
23	**0.616**	0.685	0.599	33	**0.372**	0.606	0.083
24	**0.503**	1.800	0.577	34	**0.539**	1.617	0.202
25	0.093	1.263	0.020	35	0.589	1.965	0.191
26	0.030	1.712	0.262	36	0.726	0.749	0.109
27	**0.209**	1.497	0.568	37	**0.696**	1.068	0.141
28	**0.152**	1.986	0.548	38	**0.545**	2.365	0.266
29	0.086	2.660	0.210	39	**0.361**	0.867	0.031
30	0.000	0.120	0.000	40	0.000	2.803	0.573
31	0.000	0.502	0.056				
32	**0.166**	1.661	0.205				
Mean	**0.186**	**1.389**	**0.305**	**Mean**	**0.479**	**1.505**	**0.200**
SD	0.200	0.724	0.234	SD	0.219	0.760	0.157

The newborn sera were examined with the Rubella-Immunoblot for their antibody response using topographic E1, E2 and C proteins as antigens. Resulting bands were analysed by densitometry. Densitometric values of each patient's bands are given in separate columns. Mean and standard deviation are given at the bottom of each column. Except for patients 23 and 24, E2-bands of CRS patients are less frequent and had weaker densitometric values than E2-bands of asymptomatic patients. Other bands did not differ significantly.

the Rubella-IgG-Peptide-EIA showed that most of the CRS newborns lacked antibodies directed to SP 15 (linear RV E1 protein). The asymptomatic newborns had higher (i.e. normal) SP 15 antibody levels, which on further evaluation did not differ from postnatally RV infected patients. Similarily, the results of the nonreducing Rubella-Immunoblot revealed that most of the CRS newborns showed a reduced level of antibodies directed to topographic RV E2 protein. Asymptomatic newborns had higher E2 antibody levels. Again, they did not differ in their E2 antibodies from a normal postnatally infected control group.

All the patients with CRS had been exposed to RV during the first trimester of gestation, during which time the development of the fetal immune system occurs. The immune system is able to produce antibodies to an antigen stimulus only after weeks 10–12 of gestation [Yoffey and Thomas, 1964; Stites et al., 1973]. Antigen exposure before week 10 does not seem to result in a serologic immune response. Subsequently there is a selective lack of antibodies in CRS newborns, which might be linked to the immaturity of the fetal immune system. The asymptomatic newborns were mostly infected at the end of or after the first trimester

of gestation. As the fetal immune system is able to respond to antigen stimulus with antibody production at this time, the intrauterine RV infected asymptomatic newborns had normal antibody levels.

The lack of antibodies directed to topographic RV E2 protein in newborn patients had been found with previous immunoprecipitation tests [Mazancourt et al., 1986] and supports our own findings with the nonreducing Rubella-Immunoblot. However, CRS patients develop E2 antibodies later [Mauracher et al., 1993]. This suggests a slow maturation process of the immune response rather than a general inability to produce these antibodies. It appears useful to interpret the immune response results in CRS according to the period of time between the exposure to RV and the investigation of the serum.

According to the results of this study, the Rubella-IgG-Peptide-EIA and the nonreducing Rubella-Immunoblot could provide useful additional criteria for diagnosing CRS in week 22 of gestation and in newborns. Reduced levels of antibodies directed to SP 15 (linear $E1_{208–239}$) contributes to the prenatal diagnosis of CRS. Similarily, the diagnosis of CRS of the newborn could be reinforced by determining a reduced level of antibodies directed to SP 15 and to topographic RV E2 protein. However, the significant differences of our study results were based on comparisons of two patient groups; therefore the diagnosis of CRS cannot depend on the single SP 15/E2 result only. We suggest the use of both tests, the Rubella-IgG-Peptide-EIA and the nonreducing Rubella-IgG-Immunoblot, in addition to RV standard diagnostic methods during the prenatal/newborn periods. They could help differentiate between CRS and asymptomatic intrauterine RV infection and therefore may impact on the early treatment of late-onset symptoms of CRS.

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